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It was found that the pipe flow experiment are remarkably repeatable, with threshold values for bioluminescence stimulation occurring in laminar flow at about 1 dyn cm<sup>-2</sup>. An aircraft carrier moving at 18 kts is estimated to provide supra-threshold levels of hydrodynamic stimulus throughout millions of cubic meters of seawater in its wake. Preliminary multi-spectral analysis suggests that in littoral waters, for most of the time, nearly all of the stimulated wake can be detected.

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# FLOW VISUALIZATION IN THE OCEAN – IMPLICATIONS OF LABORATORY BIOLUMINESCENCE EXPERIMENTS

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## ABSTRACT

The objective of this study is to begin to assess the potential of using flow-induced bioluminescence as a method for visualizing oceanic flow fields. The bioluminescence response of dinoflagellates to quantifiable levels of flow stimulus is characterized in laminar and turbulent pipe flow. These results are used in a numerical simulation to predict the spatial extent of the bioluminescent "footprint" associated with supra-threshold levels of flow agitation in a ship's wake. Assuming an ideal multi-spectral sensor, estimates of the ratio of bioluminescent signal to ambient light noise are made for different bioluminescence potentials, flow agitation and ambient light conditions.

It was found that the pipe flow experiments are remarkably repeatable, with threshold values for bioluminescence stimulation occurring in laminar flow at about  $1 \text{ dyn cm}^{-2}$ . An aircraft carrier moving at 18 kts is estimated to provide supra-threshold levels of hydrodynamic stimulus throughout millions of cubic meters of seawater in its wake. Preliminary multi-spectral analysis suggests that in littoral waters, for most of the time, nearly all of the stimulated wake can be detected.

## I. INTRODUCTION

Awak'd before the rushing prow,  
The mimic fires of ocean glow,  
Those lightnings of the wave;  
Wild sparkles crest the broken tides,

And flashing round, the vessel's sides  
With elfish lustre lave;  
While far behind, their livid light  
To the dark billows of the night  
A blooming splendour gave

From Lord of the Isles (1815)  
By Sir Walter Scott

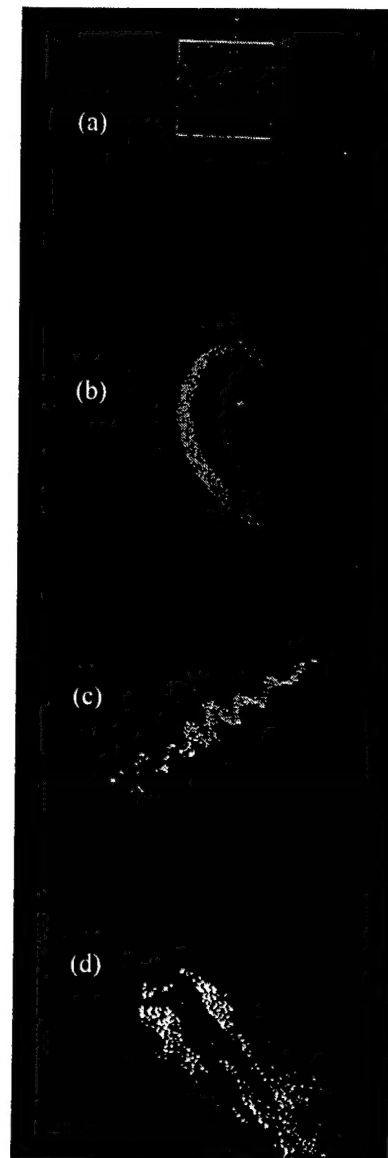


Fig. 1: Bioluminescence recordings of: (a) a sphere and (b) a rope hung from a boat, 5-6 m below the surface, with stimulation resulting from passing swell; and the bioluminescent "signature" of the wake of (c) a fish and (d) an underwater swimmer.

It is a common occurrence at night to observe bioluminescence stimulated by the highly turbulent flows associated with ship wakes. Less appreciated is the fact that much gentler flows have the capability of stimulating bioluminescence. The video frames in Fig. 1 were recorded with a low light camera and are illustrative of the bioluminescent displays visible to the naked eye, even when the stimuli are relatively weak. In Figures 1a and b the outline of a 0.25-m diameter sphere and the configuration of a rope are discernable several meters beneath the sea surface as a result of the bioluminescence stimulated by passing swell. The total bioluminescence flux from the 0.25-m diameter sphere was estimated to be about  $2.9 \times 10^{11}$  photons/s (Rohr *et al.*, 1994). Figures 1c and d contain images representative of the bioluminescent signatures of swimming fish and divers.

The organisms responsible for these displays are primarily dinoflagellates, unicellular marine plankton which are typically the most abundant sources of bioluminescence in near-surface, coastal waters (Staples 1966, Morin 1983, Lapota 1994). Seliger *et al.* (1969) have observed for the dinoflagellate *Pyrodinium bahamense* that a concentration of one cell per ml are sufficient to observe the "white" bioluminescent wake of a boat, and concentrations of several hundred per ml provide a brilliant glow bright enough to read by. It has been suggested that concentrations of dinoflagellates as low as 100 cells per liter are sufficient for bioluminescence at night to highlight moving objects (Morin, 1983). Where extended time series monitoring of coastal bioluminescence have been conducted, bioluminescent dinoflagellate concentrations have been generally found to be greater than 100 cells per liter (Kelly, 1968; Lapota *et al.* 1994; Lapota 1998).

Before bioluminescence can be seriously considered as a possible strategy for flow visualization in the ocean, two questions must be first addressed:

- (1) Do bioluminescent organisms exhibit a repeatable, characteristic response to hydrodynamic agitation?
- (2) Under what conditions can flow-induced bioluminescence be detected?

To address question one, the principal focus of this study, the responses of cultures of the red tide dinoflagellate *Lingulodinium polyedrum* and freshly collected mixed samples of luminescent dinoflagellates were studied in fully developed pipe flow. In fully developed pipe flow the shear stress and turbulent eddy scales, believed to be important for hydrodynamic stimulus, are well characterized (Rohr *et al.* 1994; 1997). The pipe flow experiments with mixed plankton samples were conducted over the course of several years. Mixed plankton samples were collected from San Diego (SD) Bay, off San Clemente Island (SCI) and from the pier at the Scripps Institution of Oceanography (SIO). Several recurring trends were found throughout all the data. These results make it possible to estimate the spatial "footprint" of stimulated bioluminescence within any well-characterized flow field. The spatial extent of a 18 kt

aircraft carrier's wake capable of stimulating bioluminescence was, through a numerical simulation, predicted as an example and for possible comparison.

Opportunities to observe bioluminescence are greatly affected by levels of background light. In an overview of coastal bioluminescence, Morin (1983) discusses the photic environment wherein bioluminescence may be visually observed. At the sea surface, bright daylight is about six orders of magnitude greater than maximum nighttime bioluminescent intensities. The intensity of moonlight at the sea surface can vary from being two orders of magnitude greater than maximum bioluminescence to two orders less. Over the last 20 years, land-based multi- and hyperspectral technology has progressed to the point where very low contrast objects can be detected in cluttered backgrounds because of subtle differences between object and background spectra. Only recently has this technology been applied to the marine environment.

Flow-induced bioluminescence appears to be an attractive candidate for multi-spectral analysis. Emission spectra of *in situ* dinoflagellate samples from San Diego Bay have been found to peak around 480 nm (Losee and Lapota, 1981). This emission spectrum is similar to that measured from cultured dinoflagellates (Hastings and Sweeney, 1956) and is much narrower than that associated with moonlight.

A range of bioluminescence source strengths (photons/s/ml) was derived from shipboard photometer system measurements (Losee and Lapota, 1991) and video recordings (Fig. 1a) of *in situ* bioluminescence. The shipboard photometer system employed a flow agitator that provided relatively high intensity turbulent flow. As a first step towards assessing the use of bioluminescence as a tool for visualization, the bioluminescence excited in the flow agitator was considered representative of that stimulated in the upstream regions of the ship's wake where turbulent levels are maximal. In contrast, the video recordings were of a relatively low intensity turbulent flow and consequently were associated with the downstream regions of the ship's wake, where turbulence levels are minimal. Preliminary signal to noise calculations were made using this range of bioluminescence source strengths, and background night light conditions ranging from overcast to a full moon.

## II. METHODS

### A. Pipe Flow

#### 1) Pipe-flow measurements:

Fully developed pipe flow was chosen to stimulate bioluminescence because it: (1) is hydromechanically well characterized, (2) offers a wide (and sometimes overlapping) range of laminar and turbulent stimuli, (3) provides continuous replacement of luminescent organisms, and because (4) levels of bioluminescence do not appear to be significantly degraded by the organisms'

upstream transit through the pipe (Losee and Lapota, 1981). For the purpose of this study, the most important feature of fully developed pipe flow is that the shear stress at the pipe wall,  $\tau_{wall}$ , can be calculated (Schlichting, 1979) from a simple measurement of pressure drop:

$$\tau_{wall} = (\Delta P/L)(D/4) \quad (1)$$

where  $\Delta P$  is the pressure drop between pressure taps spaced  $L$  apart,  $D$  is the pipe diameter,  $\rho$  is the water density and  $U_{avg}$  is the average flow speed. This relationship is true regardless of the laminar or turbulent nature of the flow. Also independent of whether the flow is laminar or turbulent, the shear stress profile in the pipe varies linearly from zero on centerline to a maximum at the wall.

The primary pipe flow apparatus was designed to be portable, and consisted of a 75-liter head tank, a gently contracting inlet section, and a 0.635-cm i.d. polycarbonate pipe (Rohr et al. 1990). The head tank was filled from buckets of surface collected seawater for the mixed plankton experiments and filtered seawater for the unialgal cultures. Each experiment consisted of a single tank of seawater. Dye visualization showed that the flow through the pipe inlet always remained laminar. A valve at the end of the pipe manually controlled the flow rate. Volumetric flow rate was measured by weighing the discharge fluid collected for a known period of time. In order to check for any bias introduced by different tank residency times or lack of cell homogeneity the sequence of flow rates typically proceeded from maximum turbulent to below threshold in laminar flow, and then in reverse order from laminar to turbulent. Average flow rates were calculated by dividing the volumetric flow rate by the pipe cross-sectional area. A variable reluctance differential transducer (Validyne Corporation) measured pressure drop along the pipe. Wall shear stress values were calculated from (1).

In order to determine if the pipe flow was fully developed, laminar, turbulent, or transitional, the Darcy friction factor:

$$\lambda = [\Delta P(D/L)] / (1/2 \rho U_{avg}^2), \quad (2)$$

and the Reynolds number:

$$Re = U_{avg} D / \nu, \quad (3)$$

were calculated and compared with accepted values. Excellent agreement was generally found (Fig. 2). However some discrepancy was evident for laminar flows (as determined by dye visualization) at Reynolds numbers greater than 3000, where the Darcy friction factors were noticeably higher than the theoretical values. This discrepancy resulted because the pressure tap locations, 95 and 146 pipe diameters downstream of the pipe inlet, were not sufficiently downstream to ensure fully developed laminar flow at higher Reynolds

numbers. Corresponding  $\tau_{wall}$  calculations will be higher than predicted for laminar flow, e.g. about 40% at  $Re = 6000$ .

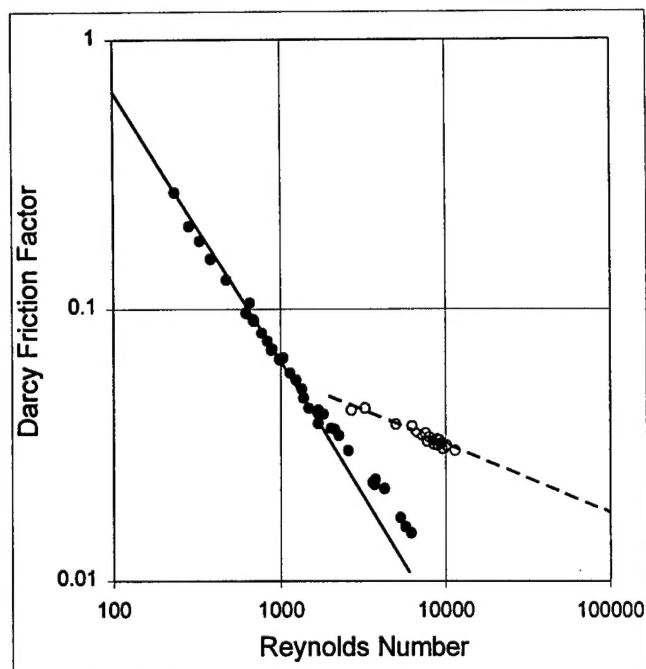


Fig.2 Representative measurements of Darcy friction factor and Reynolds number. The solid line is the theoretical relationship for laminar flow; the dashed line is the accepted empirical relationship for turbulent flow (Schlichting, 1979). Data are from the 9/20-21/93 experiments performed in the 0.635-cm i.d. pipe with cultures of *Lingulodinium polyedrum*. Closed and open symbols represent laminar and turbulent flows respectively.

## 2) Bioluminescence measurements:

Light emission was measured using two photon-counting, RCA model 8575, photomultiplier tubes (PMTs). The face of each PMT was attached to an opaque housing clamped directly onto the pipe. With this arrangement, the PMT field of view encompassed 5.0 cm of pipe length and the entire pipe diameter. One PMT was always positioned near the bottom of the pipe, 67-cm ( $\approx 106$  pipe diameters) from the inlet. A second PMT was positioned sometimes near the top of the pipe, 6-cm ( $\approx 9$  pipe diameters) from the inlet; near the middle of the pipe, 22-cm ( $\approx 35$  pipe diameters) from the inlet; or occasionally opposite the "bottom" PMT in order to compare sensitivities. A lack of time correlation between the top and bottom PMTs demonstrated that downstream bioluminescent flashes were not stimulated at the inlet, nor was light being internally reflected down the pipe.

Bioluminescence time series were obtained at constant flow rates providing unchanging levels of  $\tau_{wall}$  for periods typically between 20 – 100 s. The longer time series were collected for the slower flow rates so that the flow volume for each time series was similar. Bioluminescence time series were normally composed of consecutive 0.005-s integrations (sampling rate of 200 Hz), that could nominally resolve individual flash events.



Mean bioluminescence levels were obtained by averaging the light emission per second over each time series, and peak bioluminescence was determined by taking the highest 0.005-s integration value. Peak bioluminescence levels were generally indicative of the flash peak of individual organisms.

Bioluminescence measurements are presented in the form of counts per second (or when calibrated, photons per second), as opposed to counts per volume. Threshold levels of  $\tau_{\text{wall}}$  are better displayed when PMT levels are expressed as counts per second. It is also more appropriate to express peak bioluminescence levels, which are indicative of individual flash peaks, as counts per second. However, expressing mean PMT levels as counts per second may obscure the interpretation of why they change with increasing flow rate. An inherent ambiguity results because both convective and stimulative effects may influence PMT levels (Seliger *et al.* 1969; Widder *et al.* 1993; Rohr *et al.* 1994).

For the later experiments (1994-95) a calibration for each PMT-pipe configuration was achieved by filling a short pipe section and a 1 ml fluid sample vial with a chemiluminescent fluid from Cyalum<sup>®</sup> lightsticks (American Cyanimid Company). Simultaneous measurements were made of the pipe PMT and the quantum emission of the 1-ml sample. Quantum emission was obtained with a Quantalum 2000 (Zefaco Inc.) calibrated photometer. This procedure provided an estimate of the photon flux at the PMT.

#### 3) At-sea experiments – San Clemente Island (SCI):

Preliminary experiments using the 0.635-cm i.d. pipe were performed at-sea on the USNS *De Steiguer*. The purpose of these early experiments was to provide an indication of whether luminescent organisms would exhibit a repeatable, characteristic response to quantifiable levels of shear stress. The *De Steiguer* was anchored each night a few miles off the southeast coast of SCI (32° 47.6'-56.1' N, 118° 25.1'-25.5' W). Twelve pipe-flow experiments were conducted between 3/7/92 and 3/11/92 over the hours of 17:30 and 04:00 in a dark room. Two PMT's were employed throughout these experiments, located 22 cm and 67 cm from the pipe inlet. Because simultaneous mean bioluminescence measurements showed good agreement, they were averaged. The sources of bioluminescence were not identified. However, based on the extended time series gathered off SCI in subsequent years (Lapota, 1998), it is assumed that the predominant sources of bioluminescence were several species of the dinoflagellates *Protoperdinium*, *Lingulodinium*, *Ceratium*, and occasionally *Noctiluca*.

#### 4) Experiments with *Lingulodinium polyedrum* cultures:

Using cultures of a single luminescent dinoflagellate at known concentrations, it was possible to better assess the luminescent response of a dinoflagellate to quantifiable levels of shear stress. Experiments were

performed in the same 0.635-cm id pipe, on two consecutive days with concentrations of *Lingulodinium polyedrum* Stein of 6.5 and 6.7 cells per milliliter. The cells were grown in f/4 medium (Guillard and Ryther, 1962) minus silicate and maintained at 20±0.5 C in a Percival environmental chamber on a 12:12 hr light dark cycle. Daytime illumination levels were 240  $\mu\text{E m}^{-2} \text{s}^{-1}$  as measured with a Biospherical Instruments QSL-100 scalar radiance meter. Cell abundance was determined by counting samples under a dissecting microscope. Two hours prior to the end of the day phase, when cells are mechanically inexcitable (Biggley *et al.* 1969), aliquots from cultures exhibiting exponential growth were added to 0.45  $\mu\text{m}$  filtered seawater in the head tank. To promote cell homogeneity throughout the tank it was gently prestirred at a rotation rate of 0.5 rpm, that did not stimulate bioluminescence. Such gentle prestirring has been found to have no significant effect on response threshold (Latz and Rohr, 1999). At the beginning of the dark phase the apparatus was covered with opaque sheeting. Testing commenced 2.5 hr into the dark phase when the bioluminescence of *L. polyedrum* is maximal (Biggley *et al.* 1969). Mean and peak bioluminescence measurements were obtained at the bottom PMT, 67 cm from the pipe inlet.

#### 5) Comparison experiments – SD Bay vs SIO pier:

Twelve 0.635-cm id pipe flow experiments were performed over a year (5/30/94-7/11/95) with mixed plankton samples collected from SD Bay (6 experiments) and off the pier at SIO (6 experiments). Samples were collected and the head tank filled about 1-2 hrs before sun set. The apparatus was covered at sunset and experiments commenced about 2 hrs later. The purpose was to again test the response repeatability of luminous organisms to quantified levels shear stress, but now over a year and at two different locations. To identify species and determine abundance of the luminescent organisms present during the experiments, sea water samples were first preserved in a solution of 1% glutaraldehyde and 1% paraformaldehyde. Samples of 10 to 25 mls were poured into a settling chamber (Aquatic Research Instruments) and allowed to settle between 2 h and 24 h. No differences in population counts were noted as a function of settling time. Total numbers of organisms were counted at 100x magnification using an inverted microscope. Mean and peak bioluminescence measurements were obtained at the bottom PMT, 67 cm from the pipe inlet.

#### B. Ship Wake Simulation

The numerical wake simulation model, TBWAKE, estimated the "bioluminescent footprint" of an aircraft carrier (Table 1.) moving at 18 kts ( $\approx 9 \text{ m s}^{-1}$ ). The evolution of this numerical model has been well documented and verified with full-scale experiments on many classes of U.S. Naval surface craft (Hyman, 1990;

Hyman, 1994). The code is a parabolic Navier-Stokes solver in which the equations describing the instantaneous flow field are ensemble averaged. The result is a set of three-dimensional differential equations that are elliptic in character. If the assumption is made that the streamwise pressure and stress gradients are negligible and that there is no flow reversal, then terms which render the equations elliptic can be neglected and the equations become parabolic. Unfortunately the simulation can not be initiated very close to the ship, but rather at some distance downstream where the above assumptions are satisfied. At that location, all dependent variables must be specified, forming what will be termed the Initial Data Plane. The numerical algorithms then propagate that data downstream. Flow data comprising the Initial Data Plane is generated by solving the full Reynolds averaged Navier-Stokes equations to determine the flow around the ship and into the near wake. A  $k-\epsilon$  turbulence model together with the algebraic stress turbulence model of Rodi (1982) was used to achieve closure.

The simulation proceeded from 165 m (0.5 ship length) to 6765 m (20.5 ship lengths) downstream of the aircraft carrier. The computational domain extends out to 0.5 ship lengths horizontally from the wake centerline. Normally the code computes three mean flow velocity components, pressure, and turbulence quantities on a series of two-dimensional planes at increasing distances astern. Based on the pipe flow results, the simulation included within the wake where the flow was stimulatory to bioluminescent dinoflagellates.

Len. (m)	Beam (m)	Draft (m)	Disp. (tons)	No. of Prop.	Prop. Dia. (m)
332.5	40.2	11	91000	4	6.4

Table 1. Waterline ship geometry for aircraft carrier.

### C. Estimating Bioluminescence Source Strength

#### 1) General strategy:

The pipe flow experiments were intended to identify a shear stimulus threshold and general bioluminescence response pattern for dinoflagellates. Because of advection effects, the pipe flow experiments cannot provide unambiguous estimates of bioluminescence levels in the wake (Rohr *et al.* 1994). As a first order approximation towards bracketing the bioluminescence source strength (photons/s/ml<sup>3</sup>) throughout a ship's wake, the following strategy was adopted.

First, it was assumed (assumption 1) that the bioluminescence source strength measurements obtained from the turbulent flow agitator of the Losee and Lapota (1981) shipboard photometer system were representative of maximal values (for a given bioluminescence potential). As such, these bioluminescence measurements should not increase significantly with increased flow agitation. They should then also be indicative of the bioluminescence source strength of highly turbulent regions of the upstream wake

of a vessel moving through waters of the same bioluminescence potential.

To approximate the bioluminescence source strength associated with the downstream edge of the wake, where hydrodynamic forces are near background, the bioluminescence recording of the sphere in Fig. 1a was used. The recording was made with an intensified silicon intensified target (ISIT) video camera (Cohu Inc., model 5162). The sphere was a 0.25-m diameter Benthos glass float wrapped in black shade cloth and suspended from a black electrical cable (1.4 cm in diameter) with a weight suspended below it. The float was lowered from the ship's winch to depth of about 6 m. The recording was obtained on the night of 2/26/89 in the Southern Gulf of California about 40 miles off La Paz, Mexico. The sky was clear with no moon, and there was little or no wind. The surface water was smooth with a slight swell and the depth was on the order of 1000 m. As the ship gently rocked with passing the swell, the sphere would slowly move up and down with an amplitude estimated to be about 0.5 m.

Bioluminescence source levels were estimated at  $2.9 \times 10^{11}$  photons/s/ml (Rohr *et al.* 1994). Based on observation, the volume where stimulated bioluminescence occurred around the sphere was estimated to be about two times the sphere volume or  $1.3 \times 10^5$  cm<sup>3</sup>. Dividing source level by stimulated volume results in an average bioluminescence source strength of about  $2.2 \times 10^6$  photons/s/ml. Examination of samples of seawater collected during this time revealed the presence of the bioluminescent dinoflagellates *Lingulodinium*, *Ceratium* and *Pyrocistis*.

During the time the video recordings of the sphere were taken, the same photometer system of Losee and Lapota (1981) was measuring maximum bioluminescence source strength. A level of  $10^8$  photons/s/ml, typical of the area, was measured. The ratio of bioluminescence source strengths obtained in the relatively high turbulent flow in the viewing chamber and the relatively low turbulent flow around the sphere was about 50. It is assumed (assumption 2) that this ratio between maximum and minimum bioluminescence source strengths for different turbulent flows should be roughly preserved for different concentrations and assemblages of bioluminescence dinoflagellates.

#### 2) Test of assumptions – flow agitator:

In order to test the previous assumptions (1 and 2), a second pipe flow apparatus was built to accommodate a flow agitator which was identical to that used by Losee and Lapota (1981). The 2.54-cm i.d. hose normally used for shipboard measurements was replaced with a 2.54-cm i.d. acrylic pipe that was connected through a smooth contracting inlet to a pressurized 450-liter head tank. A relatively large, pressurized head tank was required to achieve the high volume flow rates used for the field studies. The apparatus and accompanying instrumentation were enclosed in a hut built on a pier in San Diego Bay. The head tank was filled by first opening a valve to a submerged tank which drew seawater from

2 to 3 m below the surface. When the submerged tank was full it was pressurized to slowly transfer its contents into the head tank. The maximum shear stress during transport through the 10-cm i.d. connecting hose was estimated to be less than  $1 \text{ dyn cm}^{-2}$  ( $0.1 \text{ N m}^{-2}$ ).

Pressure drop measurements along the upstream pipe indicated that the flow was always turbulent and fully developed where the PMT measurements were taken, 2.4 m from the pipe inlet. Simultaneous PMT measurements were obtained in the upstream pipe and in the flow agitator, 1.2 m further downstream. The volume of flow viewed by the pipe and flow agitator PMTs were nearly the same, 25.3 cm and 25.5 cm respectively. Since the pipe and flow agitator were in series, average flow rates through them at any specific time were identical.

To investigate assumption 1 - that the flow agitator measurements were indicative of the maximal levels of mechanically stimulated bioluminescence (per unit volume) possible, flow rates up to 2.1 L/s were tested in the flow agitator. This was about 6 times the 0.34 L/s flow used by Losee and Lapota (1981). To test assumption 2 - that the ratio of bioluminescence source strengths (photons/s/ml) between two different levels of turbulent flows is approximately independent of plankton abundance and concentration, six pipe-flow/agitator experiments were run throughout the year (6/23/94-3/30/95) during which the plankton abundance and concentration varied.

### III. RESULTS

#### A. Pipe Flow

##### 1) At-sea experiments - San Clemente Island (SCI):

Dark noise for both mid and bottom PMT's was between 100 and 200 counts per second. Mean bioluminescent measurements collected at the same time generally exhibited good agreement and consequently were averaged. During the course of these 12 experiments, performed over 5 nights, transitional Reynolds numbers varied between 3500 to 7600. As a consequence, the composite plot of mean bioluminescence as a function of wall shear stress,  $\tau_{\text{wall}}$ , does not show a characteristic break as it would for a single transition. Instead between  $\tau_{\text{wall}}$  values of 20 and  $30 \text{ dyn cm}^{-2}$  both laminar and turbulent flows overlap.

Overall a distinguishable, repeatable trend was found among the 246 measurement pairs of mean bioluminescence and  $\tau_{\text{wall}}$  measurements (Fig. 3). For  $\tau_{\text{wall}}$  greater than around  $20 \text{ dyn cm}^{-2}$  the average bioluminescence levels, expressed as counts per second, increased approximately as the first power of  $\tau_{\text{wall}}$  (slope = 0.82,  $R^2 = 0.72$ ). For  $\tau_{\text{wall}}$  less than about  $20 \text{ dyn cm}^{-2}$  this slope was noticeably steeper (Fig. 3). Unfortunately the valve used at that time could not

provide constant flow rates for flows where  $\tau_{\text{wall}}$  was less than  $7 \text{ dyn cm}^{-2}$ . The sources of bioluminescence were not identified. Based on the extended time series gathered off San Clemente Island in subsequent years (Lapota, 1998), it is assumed that the predominant sources of bioluminescence were several species of the dinoflagellates *Protoperdinium*, *Lingulodinium* and *Ceratium fusus*.

##### 2) Experiments with *Lingulodinium polyedrum* cultures

PMT dark noise was about 50 counts per second. With a new valve arrangement, lower flow rates could be maintained providing steady  $\tau_{\text{wall}}$  values to a fraction of a  $\text{dyn cm}^{-2}$ . The transition Reynolds number varied between the two experiments so that for  $\tau_{\text{wall}}$  values between about 10 and  $20 \text{ dyn cm}^{-2}$  laminar and turbulent flows overlapped.

For both experiments, a shear stress threshold between 1 and  $2 \text{ dyn cm}^{-2}$  for mean and peak bioluminescence was distinctly evident (Fig. 4). Between about 2 and  $10 \text{ dyn cm}^{-2}$ , mean bioluminescence levels increased in laminar flow as  $\tau_{\text{wall}}$  to the 1.52 power ( $R^2 = 0.70$ ) on 9/20/93, and as the 1.65 power ( $R^2 = 0.78$ ) on 9/21/93. Where the flow was turbulent, for  $\tau_{\text{wall}} > 10 \text{ dyn cm}^{-2}$ , mean bioluminescence values remained nearly constant for both experiments. Peak bioluminescence levels exhibited a graded response between 2 and  $10 \text{ dyn cm}^{-2}$  and remained essentially constant at higher values of  $\tau_{\text{wall}}$ .

##### 3) Comparison experiments - SD Bay vs SIO pier:

Consistent trends in flow-stimulated bioluminescence were found between all the experiments, despite two different sampling locations, time of year, and plankton abundance. With increasing laminar flow, mean bioluminescence first increased above background levels at  $\tau_{\text{wall}}$  values equal to 1 -  $2 \text{ dyn cm}^{-2}$  (Fig. 5a). For higher flows but still laminar,  $1 \text{ dyn cm}^{-2} < \tau_{\text{wall}} < 29 \text{ dyn cm}^{-2}$ , the SD Bay PMT data increased as the 1.77 power of  $\tau_{\text{wall}}$  (108 measurements) and the SIO pier PMT data increased as the 2.08 power (114 measurements). Combining both data sets the best fit was to the 1.75 power ( $R^2 = 0.90$ ). The gap in the data marked the transition from laminar to turbulent flow. In turbulent flow the relatively small range of  $\tau_{\text{wall}}$ ,  $61 \text{ dyn cm}^{-2} > \tau_{\text{wall}} > 218 \text{ dyn cm}^{-2}$ , and large scatter in the mean bioluminescence data, precluded the identification of a meaningful power law relationship. The corresponding peak bioluminescence levels exhibited a different dependence on  $\tau_{\text{wall}}$  (Fig. 5b). Individual flash peaks increased abruptly around  $\tau_{\text{wall}} = 1 \text{ dyn cm}^{-2}$ , continued to increase but at a slower rate until about  $\tau_{\text{wall}} = 10 \text{ dyn cm}^{-2}$ , and then remained nearly constant thereafter.

The bioluminescent organisms that were identified were exclusively dinoflagellates, specifically



*Lingulodinium polyedrum* Stein, *Ceratium fusus* (Ehrenberg) Dujardin, *Protoperidinium* spp. and *Noctiluca scintillans* (Macartney) Ehrenburg (see Table 2.). These luminescent plankton are common to the coastal waters of Southern California (Sweeney, 1963; Holmes *et al.* 1967; Lapota *et al.* 1994; Lapota 1998).

Dinoflagellate	Cell Size ( $\mu\text{m}$ )	Bioluminescence potential (quanta/cell)
<i>Lingulodinium polyhedrum</i>	40	$10^8$ (a)
<i>Ceratium fusus</i>	340x30	$5 \times 10^8$ (b)
<i>Protoperidinium</i> spp.	70x60	$3 \times 10^9$ (c)
<i>Noctiluca scintillans</i>	800	$9 \times 10^{10}$ (d)

Table 2. Size and bioluminescence potential of luminescent dinoflagellates collected during the course of the pipe flow experiments. Bioluminescence potential was estimated from previous studies where cells were mechanically stimulated until bioluminescence was depleted: (a) Biggley *et al.* (1969); (b) Esaias *et al.* (1973); (c) Lapota *et al.* (1989); (d) Buskey *et al.* (1992).

## B. Ship Wake Simulation

For the computational simulation, a  $\tau_{\text{wall}}$  value of 10  $\text{dyn cm}^{-2}$  was chosen as the cut-off for bioluminescence stimulation within the wake of the aircraft carrier that was moving at 18 kts. This value is an order of magnitude greater than the shear stress threshold for bioluminescence stimulation found in the pipe flow experiments (Figs.4a, 5a.). Consequently the spatial extent of the bioluminescent "footprint" predicted is considered conservative. A value of 10  $\text{dyn cm}^{-2}$  was also chosen because at higher shear stress levels the response of individual bioluminescent organisms remained nearly constant (Fig. 4b, 5b).

Between 0.5 (165 m) and 20.5 (6765 m) ship lengths, the depth of the wake increased from about 10 to 20 m as its width increased from about 40 to 100 m. Over this computational domain the total volume of wake with shear stresses greater than 10  $\text{dyn cm}^{-2}$  was about  $2 \times 10^7 \text{ m}^3$ . More than 98% of this volume was entrained and was never in the immediate vicinity of the propeller. The entrained water will bring in a fresh supply of organisms, which is desirable since organisms in the immediate proximity of the propellers may be depleted of their bioluminescence potential.

## C) Estimating Bioluminescence Source Strength

### 1) Test of assumptions – flow agitator

Initial tests in the flow agitator indicated that mean bioluminescence measurements remained essentially constant from about 0.2 L/s to the highest flow rate attainable, 2.1 L/s. A series of 6 additional experiments were performed during the year, for a flow range between 0.38 and 2.1 L/s. Over this range the flow in the upstream 2.54-cm id pipe was always turbulent

Plotting simultaneous mean bioluminescence measurements obtained from the upstream pipe and downstream agitator as a function of the pipe wall shear stress, several distinctive trends were evident (Fig. 6). The bioluminescence measurements obtained from the flow agitator remained nearly constant, whereas the pipe flow bioluminescence increased dramatically with  $\tau_{\text{wall}}$  (proportional to  $\tau_{\text{wall}}^{0.95}$ ;  $R^2 = 0.8$ ). Moreover, the bioluminescence levels measured in the pipe were always a small fraction of that measured in the flow agitator, increasing from about 1% to 10% over the entire range of flows. Nevertheless, at any fixed  $\tau_{\text{wall}}$  value, relative bioluminescence levels measured in turbulent pipe and agitator flows were approximately preserved.

Note, because the pipe and flow agitator are in series, it is possible that at sufficiently high levels of stimulation, a significant fraction of the total bioluminescence available may be depleted before reaching the flow agitator. The common dinoflagellate *Lingulodinium polyedrum*, for example, may only flash a few times during their entire night phase (Latz and Lee, 1995).



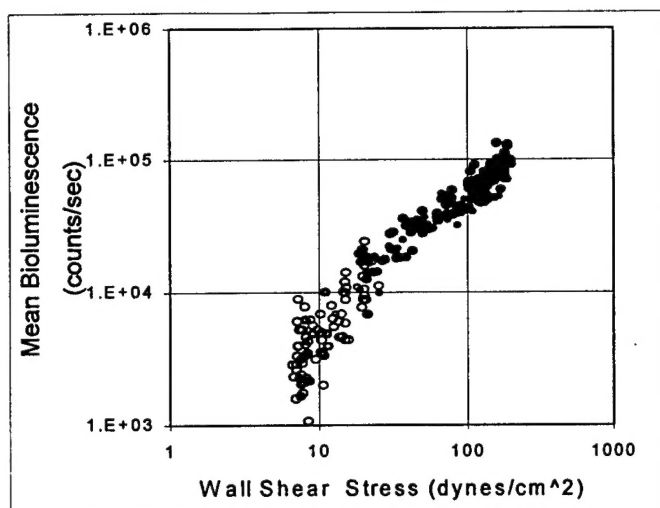


Fig. 3: Mean bioluminescence measurements obtained from mixed plankton samples collected off San Clemente Island in the 0.635-cm i.d. pipe. Open symbols represent laminar flow, closed symbols turbulent.

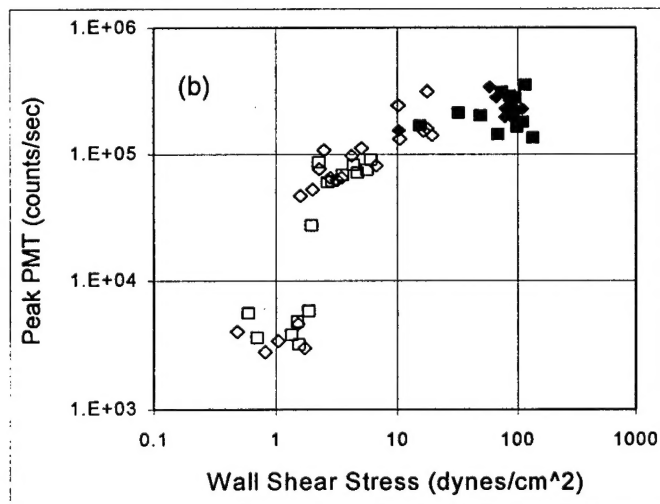
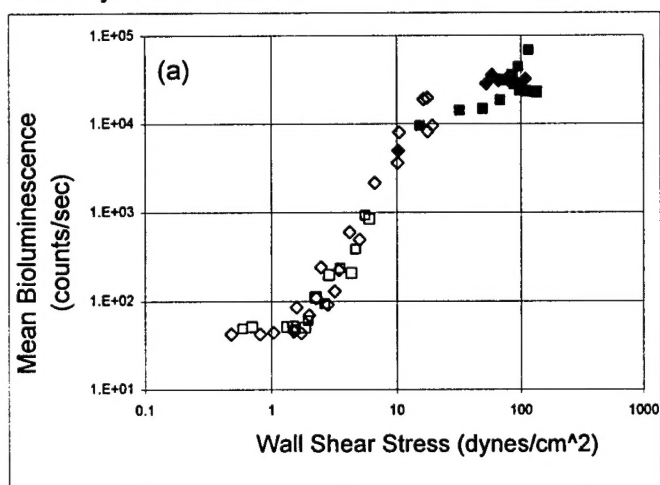


Fig. 4: Mean (a) and peak (b) bioluminescence measurements obtained from laboratory cultures of *Lingulodinium polyedrum* in the 0.635-cm i.d. pipe. Open symbols represents laminar flow, closed symbols turbulent flow; diamond symbols represent measurements collected on 9/20/93, squares 9/21/93.

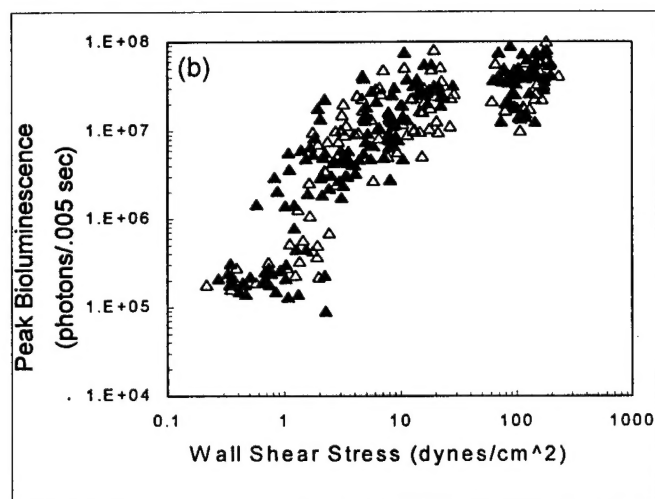
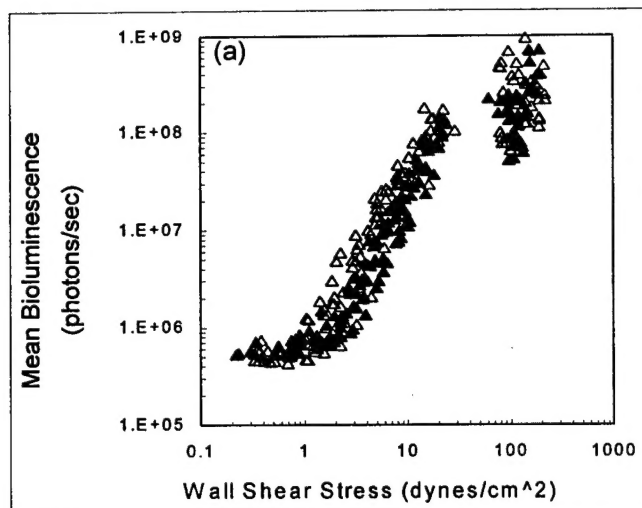


Fig. 5: Mean (a) and peak (b) bioluminescence measurements obtained from mixed plankton samples in the 0.635-cm i.d. pipe. Samples were collected from S.D. Bay (closed triangles) and off the S.I.O. pier (open triangles) over one year.

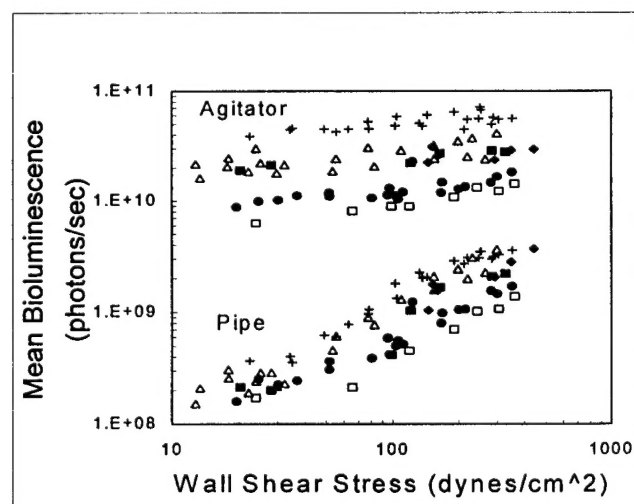


Fig. 6: Simultaneous mean bioluminescence measurements obtained from the upstream 2.54-cm i.d. pipe and the downstream flow agitator plotted as a function of the pipe wall shear stress.

## IV. DISCUSSION

### A. Pipe Flow

Bioluminescence intensity will depend on the species abundance and concentration of luminescent organisms present (Lapota, 1998), as well as their agitation (Latz *et al.* 1987), light and nutrient prehistory (Seliger *et al.* 1969; Seliger and Biggley, 1982; Swift *et al.* 1995; Sullivan and Swift, 1995). Considering this, it is particularly remarkable that throughout all the measurements, collected over several years and at various locations, there are several consistent features found. These include: a bioluminescence response threshold around  $1 \text{ dyn cm}^{-2}$ , occurring in laminar flow; a reduced rate of increase of mean bioluminescence for  $\tau_{\text{wall}} > 10 \text{ dyn cm}^{-1}$ ; and a near constant level of peak bioluminescence for  $\tau_{\text{wall}} > 10 \text{ dyn cm}^{-2}$ , regardless of whether the flow is laminar or turbulent.

Threshold shear stress levels of about  $1 \text{ dyn cm}^{-2}$  for bioluminescence have been previously reported for both mixed plankton samples collected from SD Bay (Rohr *et al.* 1997), the Sargasso Sea (Latz *et al.* 1994), the eastern Pacific (Latz *et al.* 1994) and for unialgal cultures of *Lingulodinium polyedrum* (Latz *et al.* 1994, Latz and Rohr, 1999). Threshold shear stress levels of the order of  $1 \text{ dyn cm}^{-2}$  are higher than what would normally be associated with flows within most of the ocean (Pond & Pickard, 1983; Evans, 1982; Thomas & Gibson, 1990). This is in agreement with the general lack of "spontaneous" dinoflagellate bioluminescence reported there (Bowden *et al.* 1965; Widder *et al.* 1989; Buskey *et al.* 1990). The stimulation of luminescent dinoflagellates at shear stress levels of about  $1 \text{ dyn cm}^{-2}$  in laminar flow is also consistent with observed occurrences of bioluminescence, e.g. breaking waves and swimming fish (Latz *et al.* 1994) and its function as an antipredation strategy.

A reduced rate of increase in mean bioluminescence for  $\tau_{\text{wall}} > 10\text{--}20 \text{ dyn cm}^{-2}$  has been previously reported for mixed plankton samples (Rohr *et al.* 1997) and for unialgal cultures of *Lingulodinium polyedrum* (Latz and Rohr, 1999). The near constant peak bioluminescence response of individual cells (Figs. 4b, 5b) for  $\tau_{\text{wall}} > 10 \text{ dyn cm}^{-2}$  is also consistent with what has been previously reported for SD Bay mixed plankton samples (Rohr *et al.* 1997) and cultures of *Lingulodinium polyedrum* (Rohr *et al.* 1997; Latz and Rohr, 1999).

### B. Estimating Bioluminescence Source Strength

#### 1) Test of assumptions – flow agitator:

The motivation for the flow agitator studies was to correlate the shipboard flow agitator measurements of Losee and Lapota (1981) with a range of bioluminescence source strengths (photons/s/ml) representative of the near and far regions of the wake. In order to pursue this it was assumed that the bioluminescence measurements obtained from the flow agitator, expressed as photons/s/ml, were indicative of

the maximal levels that could be mechanically stimulated.

Laboratory studies showed that increasing the flow rate through the flow agitator from  $0.34 \text{ L s}^{-1}$ , its rate for shipboard measurements, to  $2.1 \text{ L s}^{-1}$  had little effect on the mean bioluminescence measured (Fig. 6). This behavior is similar to what Seliger *et al.* (1969) have reported for highly turbulent flows in an impeller pump housing. The general insensitivity to increased flow agitation found is consistent with the assumption that the shipboard flow agitator measurements were representative of maximal bioluminescence source strength.

It was also assumed that the ratio of mean bioluminescence (per unit volume), measured in flows of different turbulent intensity, would remain relatively constant independent of the dinoflagellate species abundance and concentration. This assumption provided an estimate of the far stream bioluminescence source strength, because it was found in the sphere study that the bioluminescence source strength for relatively weak turbulence was about 2% of that found in the highly turbulent agitator flow.

There is evidence to support this assumption as well.

For a constant flow rate (and upstream pipe wall  $\tau_{\text{wall}}$ ), the ratio of mean bioluminescence measured in the turbulent flow in the agitator and pipe reasonably tracked changes in plankton abundance and cell concentration throughout the year. Latz and Rohr (1999) have found for cultures of *Lingulodinium polyedrum* that mean bioluminescence at fixed  $\tau_{\text{wall}}$  approximately scaled with concentration. Good correlation ( $R^2 > 0.8$ ) between mean bioluminescence measurements obtained in turbulent pipe flow and an identical flow agitator has also been reported for mixed plankton samples collected over several weeks from the Gulf of California (Rohr *et al.* 1990).

### C. Observing ship wake bioluminescence

This next section discusses the detectability of ship wake signatures from an airborne platform using an ideal multispectral or hyperspectral sensor. The key to detecting a bioluminescent signature is to reduce the noise clutter in the image until only statistical noise remains. Although statistical noise cannot be eliminated, it can be minimized by collecting more photons, and converting a greater fraction of them to photoelectrons. This is achieved by maximizing the aperture, the integration time and the quantum efficiency. The remaining noise ideally can be reduced through the use of an appropriately tuned multispectral sensor. The optimum bands to which the sensor is tuned depend on the inherent optical properties of the water, the spectral content of the bioluminescent light and the sensor's spectral covariance matrix. One method of making a robust tunable multispectral sensor is to start with a hyperspectral sensor and bin bands appropriately for each environment. A correctly tuned multispectral sensor

will have at least one target band in which the bioluminescent signature is detected and one to three guard bands, which are correlated to the background clutter and other noise terms.

Although more rigorous optical radiative transfer models have been developed in the literature (e.g. Gordon 1987, Vasil'kov 1991), given the coarse estimations of the bioluminescence source strengths a simple model is considered appropriate. This simple model converts a source strength (photon/sec/ml) to upwelling radiance ( $W/m^2/nm/sr$ ) by assuming that one half the photons go up isotropically and two thirds are within a 20 nm band centered at 480 nm. The water leaving radiance from the target signature is calculated by propagating the source to the ocean surface using an estimated diffuse attenuation coefficient and then through the surface accounting for the change in the index of refraction and the air/sea interface transmission. The upwelling radiance is then integrated over the resolution element of the system to a depth of 20 meters (for a ship wake). The water leaving radiance is propagated through the atmosphere and sensor optics to get photoelectrons for a typical spectral sensor.

The signal to noise ratio (SNR) of a quantum noise limited system is given by:

$$SNR = 20 \log [t_{pe} / (t_{pe} + b_{pe})^{1/2}], \quad (4)$$

where  $t_{pe}$  is the photoelectrons from the bioluminescence and  $b_{pe}$  is the number of photoelectrons reflected from background. For systems with Gaussian background statistics, reasonable detection and false alarm rates are achieved when the SNR is greater than about 13 dB.

Preliminary calculations were made for the Pacific, Atlantic and San Diego Bay where photometric measurements, using the same flow agitator studied here, have been collected (Losee and Lapota, 1981). The sensor parameters are shown in Table 3. It should be noted that changing the sensor parameters can have a fairly significant impact on the SNR's calculated.

Aperture size	4	cm <sup>2</sup>
Altitude	300	m
Integration time	1.00	seconds
Quantum Efficiency	0.09	
Resolution element	4	m <sup>2</sup>
Optics transmission	0.1	

Table 3, System Parameters

The resolution element on the ocean surface was chosen as 4 m<sup>2</sup>. Average maximal bioluminescence within a 4 m<sup>2</sup> area of the wake was calculated directly from the photometric measurements. Average minimal bioluminescence in a 4 m<sup>2</sup> was, based on the sphere study, was estimated to be simply 2% of maximal. Average source strengths for different regions of the world (Losee and Lapota, 1981) are compiled in Table 4.

	Max (a) (ph/s/ml)	Min (b) (ph/s/ml)	K490 (c) (1/m)
Atlantic	8.90E+05	1.78E+04	0.05
Pacific	1.31E+06	2.62E+04	0.05
S. D. Bay	5.62E+07	1.12E+06	0.1

Table 4, Bioluminescent source strengths (a) measured by the flow agitator of Losee and Lapota (1981); (b) 2% of (a); (c) typical light attenuation lengths at 490 nm.

The background irradiance values are taken from Mobley (1994). The background photoelectrons,  $b_{pe}$ , are calculated by assuming the background irradiance is spread evenly from 400 to 700 nm and the sea surface irradiance reflectance is 0.04. Results of the calculations are summarized in Figs. 7a and b below. Immediately notable is that the coastal area represented by San Diego shows a 20 dB higher SNR than the open ocean areas represented by the Pacific and Atlantic sources, and are easily detectable even at high background irradiance levels and low agitation.

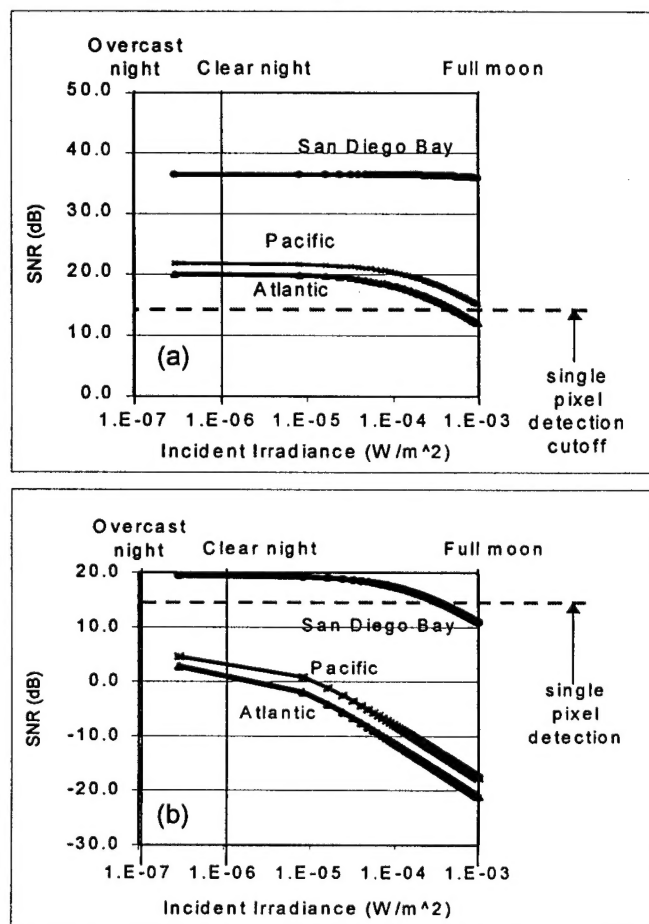


Fig. 7: Ratio of bioluminescence signal to ambient light noise for the estimated (a) brightest and (b) dimmest areas (4m<sup>2</sup>) of a ship wake.

Further study is required to assess the performance of commercially available spectral systems for detecting a variety of oceanic, flow-induced bioluminescence

fields, under a broad spectrum of ambient lighting. Sources of ambient bioluminescence noise must also be identified (e.g. breaking waves, schools of fish) and their effect on detection assessed. Nevertheless, given the pervasiveness of bioluminescent dinoflagellates, their characteristic response to shear, narrow spectral signature, and the ability for multi and hyper spectral sensors to exploit spectral anomalies, the possibility of using bioluminescence as a flow-visualization tool for oceanography is inviting.

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"Sailing at night in seas that luminesce is something splendid that is not given to all men."

Martin Wells (1998)



Fig.8. Bioluminescence image of swimming dolphin.